UNIVERSITI PUTRA MALAYSIA

THE PRESENCE OF STAPHYLOCOCCUS AUREUS ON THE SKIN, NOSE, AND EAR OF ADOLESCENT FOOTBALLERS BEFORE AND AFTER TRANING

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THE PRESENCE OF *STAPHYLOCOCCUS AUREUS* ON THE SKIN, NOSE, AND EAR OF ADOLESCENT FOOTBALLERS BEFORE AND AFTER TRAINING

By

JOHN LAURENCE WILLIAM

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

October 2004

DEDICATION

To My Late Father, Beloved Mother, Family Members, My Dearest Wife And Children Jasmeen, Josephine, Jemima, Jarvis

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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October, 2004

Chairman : Saidon Amri, PhD

Faculty : Educational Studies

The objective of the study was to determine the number of adolescent footballers carrying *Staphylococcus aureus* during outdoor and indoor training. One hundred twenty healthy male subjects age from 13 to 15 participated in the study. The subjects were assigned into two different training venues; outdoors and indoors. Swabs were taken before and after training at three sites of the body; skin, ear and nose. The strains were incubated in Baird Parker agar plates at 37°C. There was no significant difference for the numbers of *S. aureus* strains carriers between pre and post result for the skin, nose, and ear for outdoor training.

Results for the number of *S. aureus* strains carriers on the skin, and nose between pre and post indoor training showed significant difference ($p<0.05$), however no significant difference was observed for the results on the ear. Number of carriers on the skin, ear, and nose for pre training was 60 (100%), 56 (93%) and 56 (93%), respectively, whereas for post training on the same sites were 44 (73%), 56 (93%), and 60 (100%), respectively. As for the estimated mean plate count of *S. aureus* strains, there was significant difference between the pre and post results on the ear and nose of Malays during outdoor training ($p<0.05$) and no significant difference for the estimated mean plate count of strain on the skin. The estimated mean plate count of *S. aureus* strain for Malays from the skin, ear and nose before training were 138±103, 190± 147 and 395±83, respectively and after training were 97±77, 71±64 and 498±75, respectively. For Indians, there was no significant difference for the plate count of *S. aureus* strain on the skin, ear, and nose between the pre and post results during outdoor training. As for indoor training, both Malays and Indians showed a significant difference between the pre and post results ($p<0.05$). The estimated mean plate count of strain during indoor training for Malays from the skin, ear and nose before training was 33±17, 71±28 and 312±55, respectively and 21±16, 44±26 and 452±89, respectively after training. For Indians, the estimated mean plate count of strain on the skin, ear, and nose before training was 72±21, 80±21 and

309±104 respectively and 55±19, 200±62, and 466±109 respectively after training. In conclusion, the adolescent footballers are carriers during training either indoor or outdoor.

Representative strains from the skin of adolescent footballers and environment were selected randomly for antibiotic resistance, plasmid, coagulase, and RAPD-PCR analysis. For the antibiotic resistance test, nineteen antibiotics were tested. Antibiotic resistance patterns with the strains tested from footballers training indoor, outdoor and environment were diverse. However, norfloxacine (0%), rifampicin (0%), imipenem (0%), methicillin (0%) and trimethoprim-sulfamethoxazole (100%) showed a similar resistance patterns with the strains tested from footballers and the environment. Strains from the adolescent footballers training indoors and indoor environment have a higher Multiple Antibiotic Resistance (MAR) index compared from the strains from outdoor adolescent footballers and outdoor environment. The plasmid profiles of *S. aureus* strains isolated from the skin of footballers and the environment ranged between 1.8 to 3.4 megaDalton (mDa). The results of the plasmid profiles and antibiotic resistance showed that there was no correlation between plasmid carriage and resistance to a particular antibiotic tested. Thirty-two *S. aureus* strains isolated from outdoors and indoors were found to be carrying coagulase genes of different sizes. Two strains produced three

amplified coagulase gene fragments while one strain produced two amplified coagulase gene fragments. The rest of the strains produced only one amplified coagulase gene fragment. As for the strains from the environment, two strains produced two amplified coagulase gene fragments and seven produced only one amplified coagulase gene fragment. The profiles obtained from RAPD contained 1 to 9 bands within the molecular size of 0.3 kbp to 5.0 kbp. From the dendrogram, the strains were divided into 2 major clusters and at 100% similarity there were four groups of strains. It can be concluded that the strains isolated from the footballers and environment were pathogenic due to the present of the coagulase gene.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEHADIRAN *STAPHYLOCOCCUS AUREUS* PADA KULIT, HIDUNG, DAN TELINGA PEMAIN REMAJA BOLASEPAK SEBELUM DAN SELEPAS LATIHAN

Oleh

JOHN LAURENCE WILLIAM

Oktober, 2004

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Objektif kajian adalah untuk menentukan jumlah pemain remaja bolasepak pembawa *Staphylococcus aureus* semasa menjalani latihan di dalam dalam dewan dan padang. Seratus dua puluh subjek berumur di antara 13 hingga 15 tahun terlibat dalam kajian ini. Subjek-subjek di bahagikan kepada dua tempat latihan yang berbeza; di luar padang dan dalam dewan. Swabs diambil sebelum dan selepas latihan pada tiga bahagian badan; kulit, telinga, and hidung. Kesemua swabs di kulture dengan agar Baird Parker dan dieram pada suhu 37°C.

Semasa latihan di padang tidak ada kesan signifikan pada jumlah pembawa *S. aureus* sebelum dan selepas latihan pada kulit, hidung and telinga. Sebaliknya semasa latihan di dewan terdapat kesan signifikan ($p<0.05$) pada jumlah pembawa *S. aureus* sebelum dan selepas latihan pada kulit dan hidung, manakala tidak ada kesan signifikan pada jumlah pembawa pada telinga. Purata pembawa *S. auerus* pada kulit, hidung, and telinga sebelum latihan adalah 60 (100%), 56 (93%) dan 56 (93%), manakala selepas latihan ialah 44 (73%), 56 (93%) dan 60 (100%) masing-masing. Terdapat kesan signifikan jumlah anggaran *S. aureus* pada telinga dan hidung pemain Melayu semasa latihan di padang dan tiada kesan signifikan jumlah *S. aureus* pada kulit ($p<0.05$). Jumlah anggaran *S. aureus* pada kulit, telinga dan hidung adalah 138±103, 190±147 dan 395±83 sebelum latihan, dan selepas latihan adalah 97±77, 71±64 and 498±75. Untuk pemain India tiada kesan signifikan jumlah anggaran *S. aureus* semasa latihan di padang pada kulit, hidung dan telinga. Untuk latihan di dewan, terdapat kesan signifikan jumlah anggaran *S. aureus* untuk Melayu dan India pada kulit, telinga, dan hidung ($p<0.05$), Jumlah anggaran *S. aureus* sebelum latihan untuk pemain Melayu adalah 33±17, 71±28 dan 312±55, manakala selepas latihan adalah 21±16, 44±26, 452±89 masing-masing. Untuk pemain India jumlah anggaran *S. aureus* pada kulit, telinga, dan hidung semasa latihan

dalam untuk pra dan post latihan adalah 72±21, 80±21, 309±104, dan 55±19, 200±62, 466±109 masing-masing.

Strain yang mewakili dari kulit subjek dan udara di pilih secara rambang untuk ujian kerintangan antibiotik, plasmid, coagulase and RAPD-PCR. Strain dari kulit subjek dan udara diuji kerintangan antibiotik dengan sembilanbelas antibiotik. Keputusan kerintangan antibiotik untuk strain dari subjek luar, dalam dan udara mempunyai corak yang berbeza. Walaupbagaimanapun antibiotik norfloxacine (0%), rifampicin (0%), imipenem (0%), methicillin (0%) and trimethoprim-sulfamethoxazole (100%) menunjukkan corak kerintangan yang sama bila diuji dengan strain dari subjek dan udara. Nilai MAR index adalah tinggi untuk strain dari subjek dalam dan udara dalam. Secara kesimpulan strain ini adalah lebih patogenik jika dibandingkan dengan strain lain.

Keputusan menunjukkan bahawa strain dari kulit subjek dan udara mengandungi plasmid dengan saiz di antara 1.8 hingga 3.4mDa. Bagaimanapun keputusan tidak menunjukkan sebarang perkaitan di antara profil plasmid dengan profil ketahanan antibiotik tertentu. Tiga puluh dua strains dari kulit subjek semasa latihan di luar dan dalam di dapati membawa gen koagulase dengan saiz yang berbeza. Dua strain dari subjek di dapati mempunyai tiga fragmen gen koagulase and satu

strain mempunyai dua fragmen gen koagulase. Strain yang lain hanya mempunyai satu fragmen gen koagulase. Dua strain dari udara mempunyai dua fragment gen koagulase dan yang lain satu fragment gen koagulase. Keputusan profil 'fingerprinting' yang diperolehi dari RAPD mengandungi 1 hingga 9 jalur dengan saiz molekul 0.3 kbp hingga 5.0 kbp yang membentuk dua conggok utama. Pada 100% kesamaan terdapat empat conggok strain. Kesimpulannya strains daripada subjek and udara adalah patogenik kerana membawa gen koagulase.

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I certify that an Examination Committee met on 29th October 2004 to conduct the final examination of John Laurence William on his Doctor of Philosophy thesis entitled "The Presence of *Staphylococcus aureus* on the Skin, Nose, and Ear of Adolescent Footballers Before and After Training" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

JOHN LAURENCE WILLIAM

Date : 15/7/2005

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LIST OF ABBREVIATIONS

µl	Microliter
µg	Microgram
µm	Micrometer
Bp	Base pair
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
g	Gram
HCl	Hydrochloric acid
KAc	Potassium acetate
mg	Milligram
$MgCl_2$	Magnesium chloride
ml	Milliliter
mM	MilliMolar
mm	Millimeter
mol	Mole
NaCl	Sodium chloride
NaOH	Sodium hydroxide
°C	Degree celsius
PCI	Phenol-Chloroform-Isoamyl alcohol
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA

RNA	Ribonucleic acid
Rpm	Revolution per minute
SDS	Sodium dodecylsulphate
Taq	Thermus aquaticus DNA
TBE	Tris-Borate EDTA
Tris	Tris methylamine
TSB	Tryptic Soy Broth
UV	Ultraviolet
V	Volts
W/v	Weight / Volume
VO_{2max}	Maximum oxygen uptake
RBC	Red blood cell
WBC	White blood cell
NK	Natural killer cell
HDL	High density lipoproteins
LDL	Low density lipoproteins
ATP	Adenosine triphosphate
BMR	Basal metabolic rate
URTI	Upper respiratory tract infection
RPE	Rating of perceived exertion

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